Gonorrhea (*Neisseria gonorrhoeae*) and *Chlamydia trachomatis*
Nucleic Acid Amplification Technology
615-262-6362

Introduction

Screening for the presence of *Neisseria gonorrhoeae* (Gonorrhea, Gonococcus, GNDC, GC) is performed state-wide as part of a state and federally supported program to control sexually transmitted diseases. Gonorrhea affects males and females with symptoms from purulent discharge in males a few days after exposure to very mild symptoms in females. Symptoms may pass unnoticed with the consequence that an asymptomatic carrier state is formed. These carriers contribute significantly to the public health problem of gonorrhea.

Chlamydiae are bacteria that are obligate intracellular parasites of eukaryotic cells. Today *Chlamydia trachomatis* is considered the most common sexually transmitted disease (STD) in the United States. The infections it causes are among the most damaging of the STD diseases. Epididymitis is the major complication of chlamydial urethritis in men. *C. trachomatis* has been implicated in salpingitis and pelvic inflammatory disease in women.

The Tennessee Department of Health (TDH) Laboratories in Jackson, Knoxville, and Nashville accept female endocervical and urine and male urethral and urine specimens from public health clinics. These specimens are tested for gonorrhea and *C. trachomatis*. *N. gonorrhoeae* and *C. trachomatis* are identified directly from the clinical specimens by nucleic acid amplification technology (NAAT) using the Gen-Probe Aptima System.

This method is not recommended for medico-legal cases and is not acceptable for throat and rectal swabs or for antimicrobial sensitivity testing. Only culture procedures are recommended for these situations. The TDH Laboratory Services will continue to perform culture tests for *N. gonorrhoeae* under these circumstances. Antimicrobial tests are not performed in the TDH Laboratories, but can be forwarded to the Centers for Disease Control and Prevention (CDC) in special circumstances. The TDH Laboratory does not perform culture tests for Chlamydia.

ONLY SWABS SUPPLIED WITH THE NAAT SPECIMEN COLLECTION SYSTEMS CAN BE USED FOR SPECIMEN COLLECTION. The specimen must be tested within 60 days of collection, urine specimens must be tested within 30 days of collection.

This procedure is NOT for children under 12 or for medico-legal cases. Refer to the GC Culture procedure, page 45.

Specimen Collection

Female – Cervix

1. Insert a speculum into the vagina using only water as a lubricant.
2. Clean the cervical os with a swab from the kit to remove excess mucus. Discard the cleaning swab.
3. Insert a second swab from collection kit 1-1 1/2 cm into the endocervical canal, and rotate it for 30 seconds.
4. Withdraw the swab without touching the vaginal surface, and place it in transport medium.
5. Insert the blue shaft swab into the unisex Transport Tube.
6. Break off or cut the swab's shaft to fit the tube and cap the tube leaving the swab in the tube.

Gonorrhea and Chlamydia (Continued)

Female - Urethra

1. If vaginal discharge is present at urethral orifice, remove it with cotton swab before proceeding.
2. Insert a second swab 1 cm into the urethra, rotate it, remove it, and place in transport medium.
3. Insert the blue shaft swab into the unisex Transport Tube.
4. Break off or cut the swab's shaft to fit the tube and cap the tube leaving the swab in the tube.

Male Urethra

1. The patient should not have urinated for at least one hour before sample collection.
2. Collect the urethral exudate or insert the blue shaft swab from urethral collection kit 2 to 4 cm into the urethra using a rotating motion to facilitate insertion.
3. Once inserted, rotate the swab gently, using sufficient pressure to ensure the swab comes into contact with all urethral surfaces. Allow the swab to remain inserted for 2 to 3 seconds.
4. Withdraw the swab.
5. Insert the swab into the unisex Transport Tube.
6. Break off or cut the swab's shaft to fit the tube and cap the tube leaving the swab in the tube.

Specimen Identification

1. Use the established electronic information system or complete **all** the provider and patient information areas on the Chlamydia/Gonorrhea Detection Form PH-3179. Include pertinent clinical information with each specimen.

2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

Shipment of Specimens

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines of your current carrier or method of shipment.

2. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.

3. Ship the specimen to the nearest Tennessee Department of Health Laboratory in Jackson, Knoxville, or Nashville.

4. Use first-class postage on US mail.

Reporting and Interpretation of Results

Specimens are reported within 2 to 4 working days after receipt in the laboratory.

Gonorrhea results are reported
Negative for *Neisseria gonorrhoeae* by NAAT.
Positive for *Neisseria gonorrhoeae* by NAAT.
Indeterminate for *Neisseria gonorrhoeae* by NAAT. (Submit another specimen.)

Chlamydia results are reported
Negative for *Chlamydia trachomatis* by NAAT.
Positive for *Chlamydia trachomatis* by NAAT.
Indeterminate for *Chlamydia trachomatis* by NAAT. (Submit another specimen.)

In the Gen-Probe Aptima System a relative light unit (RLU) value is printed out for each specimen. Positive specimens undergo additional testing. Reports indicate that the specimen was positive, indeterminate (submit another specimen), or negative for *C. trachomatis* and *N. gonorrhoeae*.

The results of all specimens are reported to the provider who submitted the specimen. In addition, the TDH Sexually Transmitted Disease (STD) Control, the regional STD control representative, and the health department in the county where the patient lives are sent reports on positive gonorrhea and *Chlamydia* specimens.

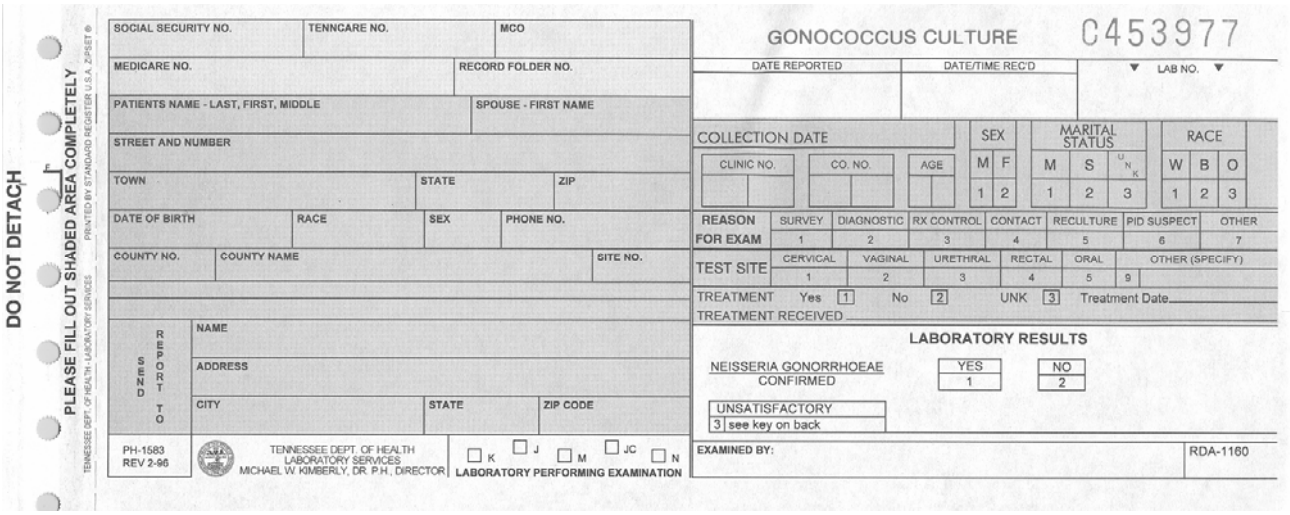
Gonorrhea and Chlamydia (Continued)

Criteria for Unacceptable Specimens

1. The specimen was not properly identified with the patient's name, the tear strip control number, or other appropriate identifier.
2. The patient identifier on the specimen did not exactly match the identifier on the form.
3. The patient was a under the age of or results for medico-legal purposes.
4. The specimen was collected by use of swabs and/or tubes (collection kit) other than by the NAAT System kit.
5. The specimen was collected from a site other than endocervical, urethral, or urine.
6. The specimen was too old for testing. All specimens should be tested within 60 days after collection (30 days for urine.)
7. The specimen had no collection swab in the transport tube upon receipt in the laboratory.
8. The specimen had two collection swabs in the transport tube.
9. The specimen was received in an out-of-date collection kit.
10. The media had leaked in transport or something has been added to the tube for (example the tube was too full or was a strange color.)

Chlamydia/Gonorrhea Detection Form PH-3179

FRONT



BACK

Unsatisfactory Key for Gonorrhea Reporting

A. Loss of Carbon Dioxide
1. No CO_2 generating tablet
2. Gono-Pak bag not sealed
3. Improper CO_2 bag used

B. Media Conditions
1. Out of Date
2. Frozen
3. Dehydrated

C. Incubation
1. Too long in transit
2. Collected and mailed on same day

D. Other
1. No specimen received
2. Improper inoculation
3. No apparent inoculation
4. No date of collection
5. _____

All specimens are incubated for a total of 72 hours and examined for typical growth. No gonorrhea was isolated. Because of the condition indicated above, this specimen is reported as unsatisfactory.

TESTING LABORATORY LOCATION CODES

J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, P.O.BOX 849, JACKSON, TN 38302-0849 - DR ORISTYNE WALKER, DIRECTOR
K = KNOXVILLE BRANCH LAB, 15622 CHEROKEE TRAIL, P.O.BOX 59019, 37950-9019, KNOXVILLE, TN - DR.M.W. KIMBERLY, DIRECTOR
N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN 37247-0801 - DR MICHAEL W. KIMBERLY, DIRECTOR

Gonorrhea, Culture Method
Neisseria gonorrhoeae, Gonococcus, GC
(615) 262-6362

Introduction

Screening for the presence of *Neisseria gonorrhoeae* is routinely performed using a nucleic acid amplification technology. Refer to the GONORRHEA AND CHLAMYDIA BY NUCLEIC ACID AMPLIFICATION METHOD, Section II. The culture method is recommended for medico-legal cases and is acceptable for throat and rectal swabs or for antimicrobial sensitivity testing. The Tennessee Department of Health (TDH) Laboratories in Jackson, Knoxville, and Nashville accept specimens from public health clinics for primary isolation of *N. gonorrhoeae*. Reference cultures are accepted from public and private health care providers for confirmation.

Gonorrhea is isolated and then identified by biochemical and direct fluorescent antibody (DFA) methods. Oxidase-positive, gram-negative diplococci (GNDC) from routine specimens are confirmed as *N. gonorrhoea* by DFA. Oxidase-positive, DFA-positive GNDC, cultures from throat cultures or children (age 12-years or less) are confirmed by a second confirmatory method. The API Quad FERM System rapid carbohydrate test is used in the TDH Laboratories.

GNDC, DFA-negative organisms from locations other than oral sites are identified using biochemicals. GNDC, DFA-negative organisms from oral sites are tested using the superoxol test. If these are positive, they are also identified biochemically.

Antimicrobial tests are not performed in the TDH Laboratories. Cultures can be forwarded to Centers for Disease Control and Prevention (CDC) for further antimicrobial susceptibility studies in special circumstances.

Specimen Collection

The culture plates, Martin-Lewis Medium (MLM), are stored sealed in plastic sleeves in the refrigerator at 2-8^{o}C. Storage life of the culture plates is six weeks. The expiration date is noted inside each sleeve. The plates should be used before the expiration date and be at room temperature before inoculation. Allow excessive moisture on the surface to evaporate before use. Dried media that has pulled away from the sides, has cracked, or has severe stress marks should not be used. Likewise, media that has growth present should not be used. Sites that may be cultured are the cervix, anus, urethra, vagina, and oropharynx. If more than one site is cultured, separate laboratory request form and culture plates are required for each culture.

Endocervical

Insert speculum into vagina using only water as a lubricant and visualize the cervix. Remove excessive cervical mucus if present. Insert cotton-tipped swab into the endocervical canal. Move from side to side. Allow 15 to 30 seconds for secretions to be absorbed.

Rectal

Insert a cotton-tipped swab 2 to 3 cm into the anal canal. If the cotton tip is inadvertently pushed into feces, use another swab to obtain a specimen. Move the swab from side to side in the anal canal. Allow several seconds for secretions to be absorbed.

Urethral

Obtain fresh exudate from meatus on sterile cotton-tipped swab, or if no exudate is present, use a calcium alginate swab inserted 2 to 3 cm into the anterior urethra.

Gonorrhea Culture Method (Continued)

Oropharynx
Swab the posterior pharynx and the region of the tonsillar crypts.

Procedure

1. Mark the bottom of the culture plate (not the lid) with the numbered tag (tear strip with accession number) from the laboratory request form.

2. Wear disposable gloves. Collect specimen from site to be cultured.

3. Roll swab in a large "Z" pattern on the culture place. (With a sterile wire loop, cross-streak immediately with a second "z" at a different angle.)

4. Place the specimen immediately into a $C0_2$ enriched environment using the $C0_2$ Tablet/Plastic Bag method.

    a. Place the culture into the bag with the $C0_2$ generating tablet within 15 minutes of inoculation.

    b. When using the individually wrapped tablet, tear the foil just enough to expose the tablet and place it in that fashion in the bag. Do not open the tablet until ready to put it into bay.

    c. Expel excess air from the bag itself; seal it tightly.

    d. Assure that no portion of the bag is left open.

    e. Incubate plates within 1 to 2 hours at 35 - 37^{o}C overnight.

5. Transport the specimen to laboratory as soon as possible to arrive within the 72-hour limit. Suggested transport is as follows:

    a. Hand deliver same day; or

    b. Incubate under appropriate temperature and $C0_2$ conditions for 18 to 24 hours. Mail the specimen to the nearest TDH laboratory.

    c. For Friday clinics proceed as for 5.a. or 5.b. mailing specimens on Saturday to arrive on Monday, or incubate all specimens except those from "STD" clinics all weekend (72 hours), and mail on Monday to arrive on Tuesday. The latter suggestion is aimed at areas having problems with specimens mailed on Saturday and delayed in transit as long as Tuesday and Wednesday. When using this alternative method (incubating 72 hours), all specimens should be clearly marked as having been incubated for 72 hours. If not marked, they will be reported as "unsatisfactory - too long in transit." Contact the laboratory before instituting this procedure.

Chart II - 4
HANDLING OF NEISSERIA GONORRHOEAE CULTURES
BY HEALTH CARE PROVIDERS
MARTIN-LEWIS PLATES

Temperature of Medium When Inoculated	ROOM TEMPERATURE. The plates should be taken from refrigerator storage at least one hour before use. Tempered plates are preferable, but specimens may be inoculated on cold plates if time does not permit tempering.
Shelf Life of Medium	Six weeks when sealed in plastic and refrigerated. Preparation and expiration dates of the medium will be indicated on package. Do not use beyond expiration date. DO NOT USE DEHYDRATED MEDIUM
Laboratory Form and Labeling the MLM Plate	Use special **Gonococcus Culture Form** PH-1583. Complete the form with all information requested. The detachable number on the form should be fixed to the BOTTOM (AGAR SIDE) of the plate. IT IS IMPERATIVE THAT THE DATE COLLECTED BE INDICATED ON THE FORM, otherwise an **UNSATISFACTORY** report will result.
Inoculation of Martin-Lewis Plates	ROLL swab on plate in "Z" pattern. ROLL THE SIDE OF THE SWAB for adequate exposure of swab to plate for transfer of microorganisms. Specimens from asymptomatic females may contain exceedingly small numbers of demonstrable gonococci. DO NOT USE THE TIP OF THE SWAB TO INOCULATE PLATE.
How Plates Should Be Cross-Streaked (Not required for the GonoPak.)	The "Z" inoculum should be cross-streaked with a sterile wire loop before incubation. This effects a greater dilution of the bacterial flora of secretions, especially from the female patient. DO NOT cross-streak the entire surface of the medium.
How Cultures Are Handled after Inoculation	The inoculated plates (GonoPak plates) should be placed into the accompanying whirlpack plastic bag. Drop a $C0_2$-generating tablet into bag. (Tear the foil. Do not remove pill from foil). Seal bag securely to prevent leakage of $C0_2$. (Moisture from plates will activate tablet.)

How Cultures Are Handled after Inoculation (Continued)	DO NOT ADD WATER TO BAG). DO NOT PUT FORM INTO BAG. ***N. gonorrhoeae*** must have a $C0_2$ atmosphere for growth. It is a fastidious microorganism that must be handled under optimum conditions to maintain its viability. Incubate plates for 16 to 24 hours at 35° to 37° C. in an inverted (bottom up) position. Allow the specimen to remain in incubator until ready to package for transport to laboratory. For LOW-RISK FRIDAY CLINIC ONLY - Specimen may be incubated over the weekend and mailed on Monday. Clearly, indicate ON THE FORM that the specimen has had 72 hours incubation. When a holiday falls on Monday, the specimens should be mailed Saturday. DO NOT HOLD SPECIMENS LONGER THAN 72 HOURS.
Shipment of Specimens	Package as you would any other specimen to be mailed. The form must be properly filled out and enclosed in the outer mailing container. Do not send any other type of specimen (blood, serum, etc.) in the package with Martin-Lewis plates. Use a GonoPak mailing label for First-class postpaid delivery to the laboratory. Please note the duration of incubation on outside container. Incubation time of weekend cultures must be indicated on the forms.

Specimen Identification

1. Complete **all** the provider and patient information areas on the Gonococcus Culture Form PH-1583. Include pertinent clinical information with each specimen.

2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the BOTTOM (AGAR SIDE) of the plate. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

Specimen Shipment

1. Incubate the specimen according to information in Chart II - 4, HANDLING OF NEISSERIA GONORRHOEAE CULTURES BY HEALTH CARE PROVIDERS.

2. Transport the specimen to arrive at the laboratory within 72 hours after collection.

3. Place the form in outer container. Place the cap on securely.

3. Affix the GONOPAK mailing label, return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label. (Postage is not required when the GONOPAK label is used.)

4. Ship the specimen to the nearest Tennessee Department of Health Laboratory **in Jackson, Knoxville,** or **Nashville**.

REFERENCE CULTURES

To submit reference cultures of *Neisseria gonorrhoeae*, transfer a well-isolated colony from the primary isolation plate to a fresh GONOPAK plate. Incubate under CO_2 overnight or until growth is visible. Place the culture in a CO_2 environmental transport system (available with the GONOPAK kit). Pack as above. Ship to the nearest TDH Laboratory.

Reporting and Interpretation of Results

Positive specimens are reported within 1 to 4 working days after arrival in the laboratory. Negative and unsatisfactory specimens are incubated for a total of 72 hours before reporting.

Positive cultures of *Neisseria gonorrhoeae* on children 12-years-old or younger are reported immediately by telephone.

Gonorrhea results are reported as
Negative: *Neisseria gonorrhoeae* not confirmed by culture.
Positive: *Neisseria gonorrhoeae* confirmed by culture.

Gonorrhea Culture Method (Continued)

Reporting of unsatisfactory specimens: Unsatisfactory specimens are examined for a total of 72 hours. Any unsatisfactory specimen that can be reported as positive, regardless of the unsatisfactory condition, will be reported as positive. (Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.) Negative unsatisfactory specimens will be reported as unsatisfactory with the reason given.

The results of all specimens are reported to the provider who submitted the specimen. In addition, the TDH Sexually Transmitted Disease (STD) Control, the regional STD control representative, and the health department in the county where the patient lives are sent reports on specimens positive for gonorrhea.

Special Reports:

Any GNDC (gram-negative diplococci) from a cervical or urethral source isolated in the TDH Laboratory that is identified as a *Neisseria meningitidis* is reported as a supplemental report. The provider is given an oral report by telephone followed by a written report.

Criteria for Unacceptable Specimens

Specimens are reported as unsatisfactory for the isolation of *Neisseria gonorrhoeae* according to the following key. (This key appears on the back of the Gonococcus Culture Form, PH-1583.)

a. Loss of Carbon Dioxide
   1) No CO_2 generating tablet.
   2) GONOPAK bag not sealed.
   3) Improper CO_2 bag used.

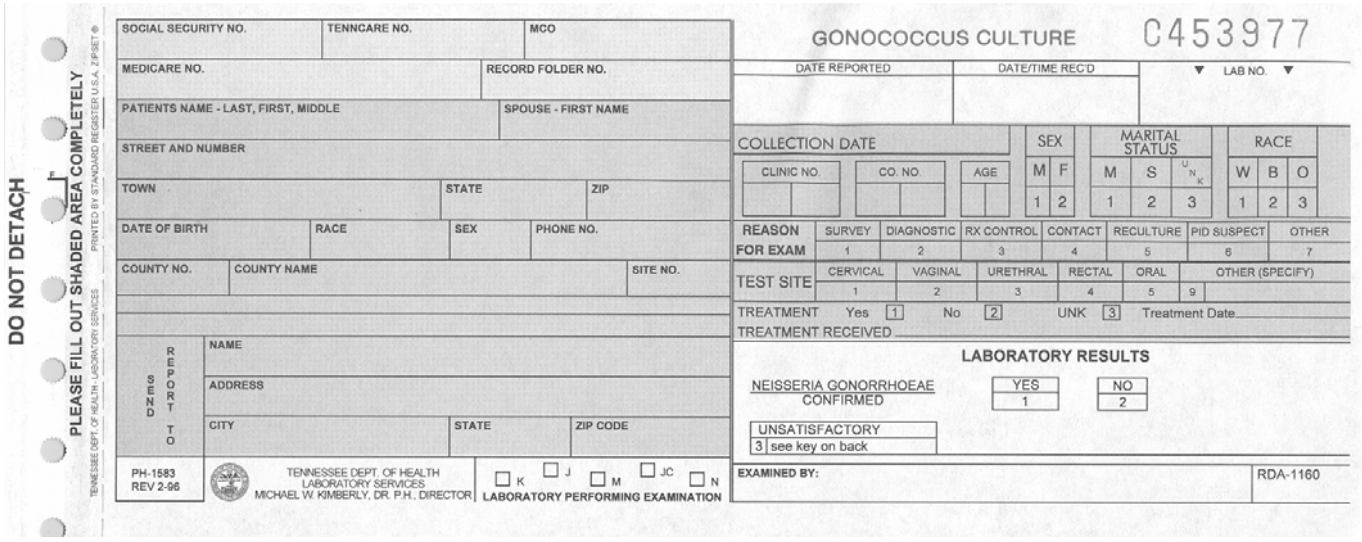
b. Media Conditions
   1) Out of date.
   2) Frozen.
   3) Dehydrated.

c. Incubation
   1) Too long in transit.
   2) Collected and mailed on same day.

d. Other
   1) No specimen received.
   2) Improper inoculation.
   3) No apparent inoculation.
   4) No date of collection.
   5) OTHER _____
      a) Overgrowth by contaminants.
      b) Failure to properly identify specimen.
      c) Broken in transit.
      d) Failure to place specimen in bag.
      e) Failure to place candle in candle jar.
      f) Laboratory accident.

Gonococcus Culture Form PH-1583

FRONT



DO NOT DETACH

PLEASE FILL OUT SHADED AREA COMPLETELY

TENNESSEE DEPT. OF HEALTH - LABORATORY SERVICES PRINTED BY STANDARD REGISTER U.S.A. ZPSET®

SOCIAL SECURITY NO. | TENNCARE NO. | MCO

MEDICARE NO. | RECORD FOLDER NO.

PATIENTS NAME - LAST, FIRST, MIDDLE | SPOUSE - FIRST NAME

STREET AND NUMBER

TOWN | STATE | ZIP

DATE OF BIRTH | RACE | SEX | PHONE NO.

COUNTY NO. | COUNTY NAME | SITE NO.

SEND REPORT TO

NAME

ADDRESS

CITY | STATE | ZIP CODE

PH-1583 REV 2-96

TENNESSEE DEPT. OF HEALTH
LABORATORY SERVICES
MICHAEL W. KIMBERLY, DR. P.H., DIRECTOR

☐ K ☐ J ☐ M ☐ JC ☐ N
LABORATORY PERFORMING EXAMINATION

GONOCOCCUS CULTURE C453977

DATE REPORTED | DATE/TIME REC'D | ▼ LAB NO. ▼

COLLECTION DATE | CLINIC NO. | CO. NO. | AGE | SEX (M 1, F 2) | MARITAL STATUS (M 1, S 2, UNK 3) | RACE (W 1, B 2, O 3)

REASON FOR EXAM: SURVEY 1 | DIAGNOSTIC 2 | RX CONTROL 3 | CONTACT 4 | RECULTURE 5 | PID SUSPECT 6 | OTHER 7

TEST SITE: CERVICAL 1 | VAGINAL 2 | URETHRAL 3 | RECTAL 4 | ORAL 5 | OTHER (SPECIFY) 9

TREATMENT Yes [1] No [2] UNK [3] Treatment Date

TREATMENT RECEIVED

LABORATORY RESULTS

NEISSERIA GONORRHOEAE CONFIRMED — YES 1 | NO 2

UNSATISFACTORY 3 see key on back

EXAMINED BY: | RDA-1160

Unsatisfactory Key for Gonorrhea Reporting

A. Loss of Carbon Dioxide
1. No CO_2 generating tablet
2. Gono-Pak bag not sealed
3. Improper CO_2 bag used

B. Media Conditions
1. Out of Date
2. Frozen
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C. Incubation
1. Too long in transit
2. Collected and mailed on same day

D. Other
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3. No apparent inoculation
4. No date of collection
5. _____

All specimens are incubated for a total of 72 hours and examined for typical growth. No gonorrhea was isolated. Because of the condition indicated above, this specimen is reported as unsatisfactory.

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